THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: 6/30/75

1. Principal Investigator (give title and degrees):

Paul Hamosh, M.D., Associate Professor of Physiology, Biophysics and Medicine

2. Institution & address:

Georgetown University
37th & O Streets, N.W.
Washington, D.C. 20007

3. Department(s) where research will be done or collaboration provided:

Departments of Physiology and Biophysics, Anatomy, Microbiology and the Georgetown University Center for the Interdisciplinary Studies in Immunology

4. Short title of study:

SMOKING AND LUNG DEVELOPMENT: A PROGRAM PROJECT

5. Proposed starting date: 1/1/76

6. Estimated time to complete: 5 years

7. Brief description of specific research aims:

An integrated, interdisciplinary approach to study the relationship between lung structure and lung function (physiological and biochemical) based on the assumption that hereditary, congenital and/or acquired (early in life) differences in geometrical patterns of the airways and lung parenchyma might be the major predisposing factor in environmental lung disease.

Three projects with major emphasis on physiology and pathology, biochemistry and anatomy (cellular and embryological) respectively, will have the following principal aims:

In Project 1, we will study the A) correlation between teenage smoking and the maturation of lung function.B) the correlation between smoking and morphometry of the lung in adolescent and young accident victims. C) The effect of mechanical stress on the pathogenesis of lung disease in experimental animals. D) the effect of smoking a single cigarette as a possible "provocation test" to separate "reactors" from "non-reactors".

In Project 2, we shall study the development of biochemical composition and function in the maturing experimental animals and the effect of smoking on biochemical development.

In Project 3, we shall study the effect of smoking on the isolated lung cells and work with genetic and "manipulated" variants with different geometrical patterns of the airways. (An abstract of all projects is on Page 5).

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OVERVIEW

P. HAMOSH M.D.

OVERVIEW

PROGRAM PROJECT

SMOKING AND LUNG DEVELOPMENT

RESPONSIBLE INVESTIGATOR: Paul Hamosh, M.D.

Historical Background. Georgetown University has recently approved the establishment of a Center for Interdisciplinary Studies in Immunology (CENTER) under the direction of Dr. Joseph Bellanti, Professor of Pediatrics and Microbiology (DIRECTOR OF CENTER). The aim of this Center is to perform coordinated research, training and clinical service with the co-operation of members, professionalsfrom different subspecialties who are distributed in the various departments of the Medical School. The nucleus for the research activities of the Center is a Pulmonary Program Project (PPP) awarded by NHLI in 1974, (HL16748). This Center has attracted many investigators, among them Drs. Hamosh, Herscowitz and Vidic, who are also grantees of the Council for Tobacco Research, Inc., U.S.A. (CTR). This resulted in gradual tightening of cooperation between these investigators with sharing of intellectual (common projects) and material resources (manpower, supplies, and equipment).

The purpose of the present program project is to expand the capabilities of the Center to achieve its goal of attracting more senior and junior investigators in the pursuit of excellence in research. The organizational interaction of all ongoing and planned functions of the Center is illustrated on Figure 1.

The proposed program project encompasses a number of projects and subprojects all tied around a central theme: Smoking and Lung Development. The working hypothesis is as follows:

Hereditary, congenital, or acquired (during maturation) factors influence profoundly lung growth and maturation. This results in a variety of geometrical patterns of lung airways and parenchyma. The geometrical structure of the lung determines its aerodynamic properties. It may well be that certain patterns of lung structure, while "physiologically" normal, may predispose to certain diseases, particularly when associated with environmental factors such as cigarette smoking and air pollution. Identifying subjects with such "predisposing lung patterns" might greatly aid in the prediction of response of the individual to environmental hazards.

The program project will be under the direction of the responsible investigator (RI) and is subdivided into three major projects centering around the physiological, biochemical and anatomical aspects of the basic theme. Each project has its principal investigator and co-investigators who are participating in several projects. The organization of the program project is illustrated on Figure 2.

Most of the projects in this program are based on previous and

present investigations funded by the CTR and to some extent by other agencies. This program is the logical outgrowth of an evolution in thought based on over two years of close work among the participating investigators. The support of the CTR, therefore, was "seed money" for further growth.

The mechanisms of concerted action will be provided by weekly meetings of the participating investigators and by pooling of information derived in these studies and from the literature. A pool of consultants will also be available. The Center provides the framework through which additional resources can be brought to play. This is especially true, considering the training and service branches, which can be tapped for additional manpower and clinical material.

The participating investigators span several departments, including Anatomy, Physiology, Pathology, Microbiology, Pediatrics and Medicine. The resources of these departments are also available for the purpose of this program project. The animal facilities of the University are fully accredited and the computational facilities are available by the courtesy of the "National Biomedical Research Foundation", a nonprofit organization affiliated with the Department of Physiology. All facilities used in this program are concentrated in two adjacent buildings, which facilitates geographically the co-operative venture (Figure 3).

The proposed program project is supplemental to the Pulmonary Programs Project which is immunologically oriented, but also emphasizes development and maturation of immuno-competence. The future cross-fertilization of these two program projects will increase the cohesiveness of the Center and establish a mechanism for attracting excellent young people for training and research.

Collaborative Arrangements

A. Within The Program Project

Each of the investigators is an expert in his field and directs a laboratory in his field. Each has (or will have) technical assistants trained to perform specific procedures. Most of the procedures described in Projects 1B,C and D, Project 2 and some in Project 3 have been performed before by the investigators. Usually a series of experiments are planned in advance, a time table prepared. Physiological measurements are performed first after which the specimens are distributed by the experimenter to the Biochemical and Morphology laboratory. Due to short distances and good communication, co-operation is proceeding smoothly.

B. Within The Center

The resources of the center are available to all investigators. At this time many of the technical personnel, paid from different sources

perform regular work for projects funded by the CTR. Of course, this is a two-way arrangement, but pooling of resources was one of the aims of the establishment of the Center.

C. Within The University

The Center and the Program Project have the support of the Departmental Chairmen, who govern the Medical School out of the Executive Faculty. The Director of the Center and the Responsible Investigator of the Program Project are tenured faculty in the Medical School. The administration of the research is enhanced by the Office of Sponsored Programs, specially instituted in 1970 to assist investigators with extra-mural support. Research on human subjects is reviewed and approved by the Research Committee and Clinical Study Committee.

D. Outside The University

Numerous collaborative arrangements are active now between the investigators in this program and investigators at other institutes in the area. To name a few: the Responsible Investigator is a part-time employee in oncology research at the local Veterans Administration Hospital. M. Hamosh worked for nine years in the National Institute of Health and as a result of it, numerous resources of the Institute are available to the group and free flow of specimens is going both ways.

A number of outside consultants will participate in advisory capacities in the Program Project. Because of technical reasons few could be contacted so far. An addendum will be sent in within the next two months with a list. Tentatively they are: Solbert Permutt, M.D. (Project 1A), Jerome Kleinerman, M.D. (1B), Donald L. Fry M.D. (1C) and Phillip M. Farrell, M.D. (Project 2).

ABSTRACT OF PROGRAM PROJECT

SMOKING AND LUNG DEVELOPMENT

Responsible investigator: Paul Hamosh M.D.

CENTRAL THEME: Developmental variations in lung structure affect susceptibility to cigarette smoke in a predictable way.

Project 1. Smoking and Functional lung maturation

Principal investigator: Paul Hamosh M.D., co-investigators: Angelo M. Taveira Da Silva M.D., Max Rabinowitz M.D. and Branislav Vidic S.D.

This project is a composite of four studies: A. The Effect of Smoking on Maximum Expiratory Flow (MEF) in Teenagers. This study is designed to answer the following questions: 1) Do changes in MEF effected by smoking differ according to sex, race and environmental factors (coincident air pollution and economic factors)? 2) Do changes in MEF over the years of development depend on the intrinsic pattern of MEF (in other words, does one pattern of MEF lead to chronic changes upon smoking, while another does not)? The study will be conducted in the Washington Metropolitan area School District in the form of annual lung functions and questionnaires in several schools located in different areas; providing therefore a diverse ethnic, environmental and socio-economic data base.

B. Smoking and "Small Airways" Disease. A Study in Pathology

A Study designed to correlate smoking history, post-mortem lung functions and lung pathology in young victims of sudden death. This study will be in co-operation with the medical examiner. It will test the hypothesis, that those subjects having pathology associated with smoking have different airway patterns than subjects who have no pathology. This study provides the morphological basis for study A.

C. Mechanical Stresses and Lung Pathology

This study will attempt to answer the question: Do mechanical forces contribute significantly to the deformation of airways and lung parenchyma and is the degree of damage related to intrinsic variation in lung structure? This will be a study in animal models, where structural changes will be induced by surgical manipulation or proteolytic enzymes. The animals with altered lungs will be exposed to mechanical lung stress. Morphometry of these lungs, when compared to controls, will provide the answer.

D. The Effect of Smoking a Single Cigarette on the "Small Airways"

This study is essentially a continuation of the study presently supported by the CTR (#878). It is an attempt to separate susceptible from non-susceptible subjects by "provocation" test, i.e. acute exposure. The question asked is: How is the response affected by ethnic and racial background 2) alteration of smoke (e.g. filters) and 3) pretreatment by pharmacological agents?

These questions are answered by sophisticated plethysmographic studies before, during and after smoking of Kentucky brand cigarettes by volunteers.

PROJECT 2

Smoking and the Biochemical Development of the Lung

Principal investigator: Margit Hamosh Ph.D., Co-investigators: T. Goeringer Ph.D., P. Hamosh M.D., B. Vidic S.D., J. Wrathall, Ph.D.

This project has two main thrusts:

1) To establish the maturation of biochemical pathways in the prenatal and postnatal period and 2) study selected enzymes the same way. The effect of exposure to cigarette smoke on the biochemistry of developing lung will be a major component of this survey. Rodents and primate lungs will be studied at various phases of their development as to their ability to incorporate radio labeled substrates for protein, carbohydrate and lipid synthesis. The biochemical findings will be correlated to functional studies with particular emphasis on lung mechanics in isolated lungs. The biochemical consequences of smoke exposure and structural alteration will also be studied.

PROJECT 3

Smoking and the Cellular and Embryological Aspects of Lung Development

Principal Investigator: Jean R. Wrathall Ph.D.
co-investigators: T. Goeringer Ph.D., M. Hamosh Ph.D. and B. Vidic S.D.

In this project, the effect of smoking and other environmental factors on the growth and development of isolated lung cells will be studied. The embryological approach is twofold: 1) To produce by manipulation of the embryo or the neonate aberrant patterns of airway development and then study the effect of smoke on the adult; and the reverse 2) study the effect of perinatal smoke exposure on development of lung structure (morphometric analysis).

While reading these abstracts might impart some superficial overview of the aims and scope of this proposal, frequent perusal of the diagramms and flowsheet is recommended. A list of recent reviews and editorials dealing with lung development follows:

1. Farrell, P.M. and M.E. Avery. Am. Rev. Resp. Dis. 111: 657, 1975.
2. Thurlbeck, W.M. Am. Rev. Resp. Dis. 111: 803, 1975.
3. Kilburn, K.H. Am. J. Med. 58: 591, 1975.
4. Symposium on Lung Growth and Development. A.T.S. Pediatric Assembly - CTS. Montreal, Canada, May, 1975 (available on tapes only).

SMOKING AND LUNG DEVELOPMENT OVERVIEW P. HAMOSH M.D.

General Introduction

The association between cigarette smoking and chronic obstructive lung disease (COLD) has been established by the famous report of the Surgeon General (1) however, to this day, this association has not been proven on a cause effect basis. In recent years, with the discovery of ever increasing numbers of other environmental agents associated with COLD(2) and the increasing realization that host factors might be as important as environmental factors(3) there is an inclination to look at COLD as a multifaceted disease. To this, one should add the realization, that smoking has become a part of our culture and the number of smokers is steadily increasing (4) therefore, one could see only two logical approaches to attempt to dissociate smoking from COLD: 1) to identify the possible harmful substances in cigarette smoke and eliminate them; 2) to identify the subjects among smokers who are at increased risk due to hereditary or acquired factors and dissuade them from smoking.

This later approach is the basis of our working hypothesis. It has been common knowledge that numerous heavy smokers live to very advanced age. Are these people "protected" from the effect of cigarette smoke by some special defense mechanism, or are the ones who develope COLD particularly "susceptible" because of some additional factors which make them vulnerable to cigarette smoke. Since the outset of our interest in the effect of cigarette smoke on the airways, we have been engaged in an intensive search for means to differentiate susceptible individuals from non-susceptible. Our original hypothesis was that there are "REACTORS" and "NONREACTORS" to cigarette smoke. We have extensively studied the effect of smoking a single cigarette on the airway responses (5) we have found that all subjects had acute bronchoconstriction and there was no bimodal distribution to justify division into two groups. Recent studies in black people (6) showed no differences in lung function between smokers and non-smokers. This fact, combined with the well known "resistance" of the black race to COLD forces us to reexamine the question on a racial basis (projects 1A and 1D).

Our original working hypothesis (1970) was based on the postulation of major differences in inervation of the large and small airways (7) this was at the time when the "small airways" (less than 2 mm in diameter) became the center of attention as the possible site of early lung disease (8) While different patterns of response involving large or small airways were shown to exist in hemp workers upon exposure to the dust (9) and differential inervation of the airways demonstrated (10) we are only now showing differences in large and small airways after exposure to cigarette smoke. These changes are not in the magnitude but rather in the time course of the response (11).

Recent investigations in humans gave us the material which serves as the foundation of our present working hypothesis: first, that it was shown that the resistance of the small airways varies widely in young human lungs (12) and the major determinants of expiratory flow were the number and diameter of airways. The second striking discovery was the reduced number of airway generations in lung from early emphysema (13). Could it be that the geometrical configuration of the airways is the primary determinant of "susceptibility" to environmental insults? Is it possible that the Shape of the conducting system of the lung determines the fate of an individual; whether he will develop COLD or not? Furthermore, if this hypothesis is true, how will one go about identifying the different shapes.

Green et al. (14) studied the maximum expiratory flow-volume (MEFV) curve in a number of healthy young subjects. They found considerable variability and comment on the possibility that one pattern of lung structure and function disposes toward a certain pathological response to a noxious stimulus, while another pattern produces a different pathology or no pathology at all. We know that structural weakness predisposes to destruction, when exposed to mechanical stresses (15), but then certain structures are more susceptible to destruction based on the design of the structure itself (e.g. suspension bridges vs. pillared ones). The complex relationship between structure, function and stresses will be explored in project 1B and 1C.

In addition to the possibility of inherent differences in patterns of lung structure (which might explain the difference in susceptibility to COLD between the white and the black race), one should also consider the environmental factors which might alter the patterns of lung structure during prenatal and postnatal development. Infection during early life is one factor, which might alter or arrest lung maturation and result in abnormal lung pattern. Exposure of the mother to noxious agents might have the same effect on the offspring. These children might grow up as "physiologically" normal subjects, while having a pattern of lung structure which marks them for trouble provided they expose this faulty patterns to environmental stresses.

The following three projects will attempt to 1) identify these patternsmorphologically, 2) to identify the functional expression of these patterns, 3) to produce different patterns in the experimental animal and challenge by environmental insults these animals.

1. Smoking and Health Report of the Advisory Committee on Smoking and Health to the Surgeon General of the Public Health Service USDHEW, 1964, Public Health Service Publication #1103.

2. Menzel, D. B., Editor. Arch. Int. Med. Symposia 9:10279, 1971.

Br. Med. J. 1:590, 1974.

Lancet 2:1134, 1973.

3. Green, G. M. Am. Rev. Resp. Dis. 102:691-703, 1970.

4. The Health Consequences of Smoking DHEW Publication #(CDC)74-8704

5. Da Silva, A. M. T. and P. Hamosh. J. Appl. Physiol. 34:361, 1973.

6. Seltzer, C. C., A. B. Siegelaub, G. D. Friedman and M. F. Collen. Am. Rev. Resp. Dis. 110:598, 1974.

7. Proposal #878 Council for Tobacco Research. P. I. Paul Hamosh, M.D.

8. Hogg, J. C., P. T. Macklem, and W. M. Thurlbeck. N. Eng. J. Med. 278:1355, 1968.

9. Bouhuys, A. and K. P. van de Woestijne. J. Clin. Invest. 49:106, 1970.

10. Cabezas, G. A., P. D. Graf, and J. A. Nadel. J. Apply. Physiol. 31:651, 1971.

11. Hamosh, P., and A. M. Taveira Da Silva. Unpublished observations.

12. Niewoehner, D. E., J. Kleinerman and D. B. Rice. N. Eng. J. Med. 219:755, 1974.

13. Henderson, R., A. Hislop and L. Reid. Thorax 26:195, 1971.

Hislop, A. and L. Reid Thorax 25:682, 1970.

14. Green, M., J. Mead, and J. M. Turner. J. Apply Physiol. 37:67, 1974.

15. Caldwell, E. J., R. D. Powel and J. P. Mullooly. Am. Rev. Resp. Dis. 102:516, 1970.

OVERVIEW

Program Project
Smoking and Lung Development

P. HAMOSH M.D.

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s)	% time	Amount
Paul Hamosh M.D.	60%	12,427
A. Taveira Da Silva M.D.	50%	14,794
Margit Hamosh Ph.D.	100%	26,053
Jean R. Wrathall Ph.D.	50%	11,000
Post-Doctoral Fellow	100%	14,202
		78,476
Technical		
Administrative Assistant	100%	11,816
6 Technicians	600%	67,597
Programmer	20%	3,906
		83,319

All Salaries Include 18.35% Fringe Benefits
(16.7% 1/1/76-6/30/76 and 20% 7/1/76-12/31/76)

Sub-Total for A 161,795

B. Consumable supplies (by major categories)

Sub-Total for B 35,000

C. Other expenses (itemize)

Sub-Total for C 25,500

Running Total of A + B + C

D. Permanent equipment (itemize)

Sub-Total for D 53,200

E

Overhead to be negotiated at 15% or higher of A,B & C

Total request 275,495

The budgets are itemized in the individual project budgets.

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First Year Budget — OVERVIEW

Central Service and Facility — P. HAMOSH M.D

3.

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
Dr. Paul Hamosh	35%	12,427
Technical		
Administrative Assistant	100%	11,816
Programmer	20%	3,906
Technician	100%	11,816
	Sub-Total	39,965
B. Consumable Supplies (list by categories)		
Office supplies, copying etc.		4,000
	Sub-Total	4,000
C. Other Expenses (itemize)		
Publication		1,000
Travel		2,000
Consultation Fees etc.		2,000
	Sub-Total	5,000
D. Permanent Equipment (itemize)		
Scintillation Counter (3 channel)		18,000
E. Overhead (15% of A+B+C)	Sub-total	18,000
	Total	66,965

Overhead to be negotiated at 15% or higher of A, B, & C

The distribution of efforts by all investigators is presented in Figure 2

10a

- GEORGETOWN UNIVERSITY CENTER FOR INTERDISCIPLINARY STUDIES IN IMMUNOLOGY

Director: Joseph Bellanti M.D. Deputy Director: Sorell Schwartz Ph.D.

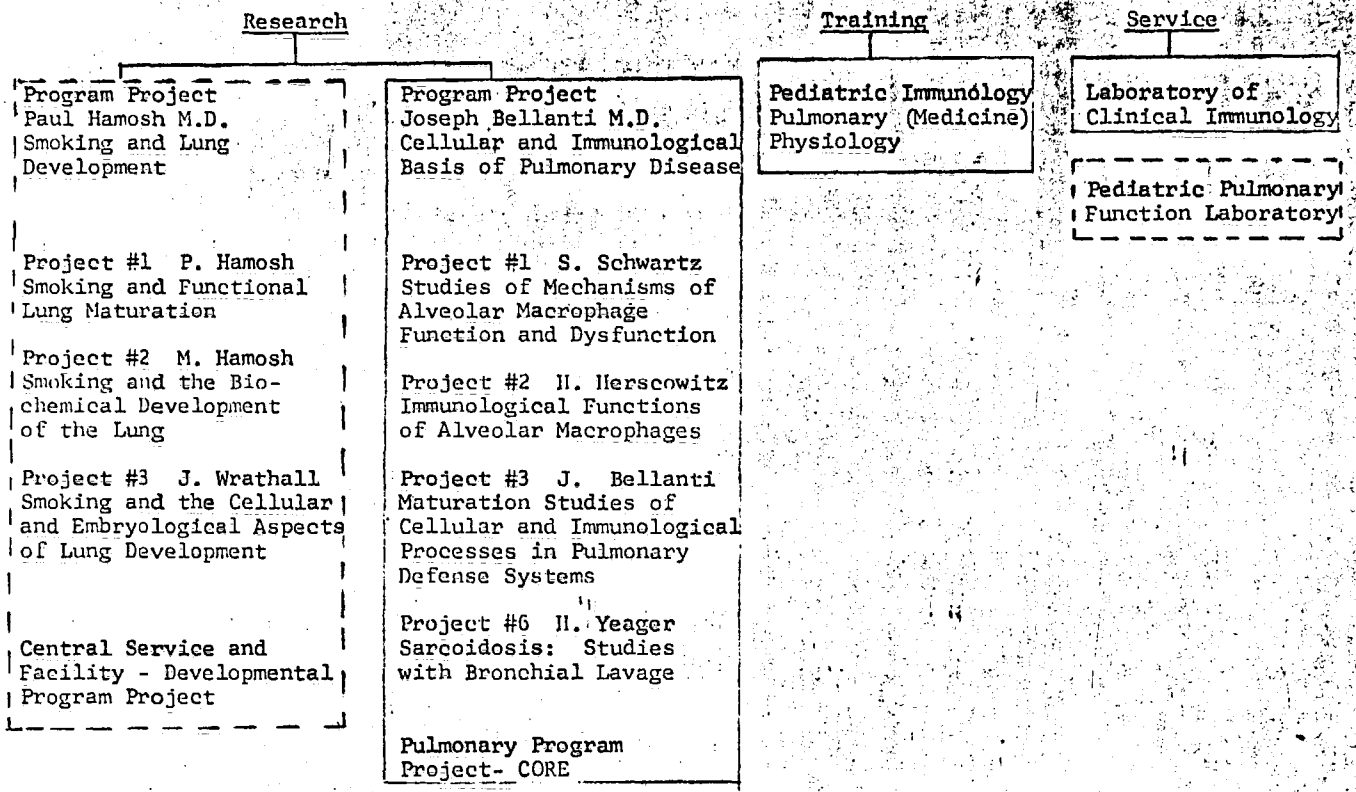


Figure 1.

OVERVIEW

P. HAMOSH M.D.

DISTRIBUTION OF EFFORT IN THE PROGRAM PROJECT

SMOKING AND LUNG DEVELOPMENT

Central Service and Facility

Responsible Investigator:	P. Hamosh	25%
Administrative Assistant		100%
Computer Programmer		20%
Technician		100%

Project #1 P. Hamosh 25%
Smoking and Functional Lung Maturation

A. The effect of teenage smoking on maximum expiratory flow
; A. da Silva 30%
Technician 100%

B. Smoking and "Small Airway" Disease: Pathology
N. Rabinowitz 30%
B. Vidic 10%
Technician 100%

C. The Effect Of Mechanical Stresses on Lung Tissue
B. Vidic 10%
Technician 100%

D. The Effect of Smoking A Single Cigarette on The "Small Airways"
A. De Silva 20%
Technician 50%

Project #2 M. Hamosh 75%
Smoking and the Biochemical Development of the Lung

Post Doctoral Fellow	100%
J. Goeringer	20%
P. Hamosh	10%
B. Vidic	10%
J. Wrathall	10%
Technician	100%

Project #3 J. Wrathall 50%
Smoking and the Cellular and Embryological Aspects of Lung Development

J. Goeringer	25%
M. Hamosh	25%
B. Vidic	10%
Technician	100%

OVERVIEW

P. HAMOSH M.D.

FIGURE 2.

DISTRIBUTION OF RESOURCES BY DEPARTMENTS

Physiology	Anatomy	Pathology	Microbiology	Pharmacology
P. Hamosh *	B. Vidic *	M. Rabinowitz	H. Hezscowitz *	S. Schwartz *
A.T. da Silva	J. Goeringer	technician		
Adm. Assist.	M. Hamosh			
technician	J. Wrathall			Pediatrics
	Post-Doc. Fellow.	Histopathology		
Pulmonary Function	2 technicians	Laboratory		J. Bellanti *
Laboratory			Smoking Machine	
Physiology Lab.	Microscope Suite		Immunology Lab.	
Office Headquart.	Biochemistry Lab.			Medicine
Computer terminal	Tissue Culture Suite			H. Yeager *
	Embryology Lab.			(P. Hamosh)
Vivarium	Radioisotope Lab.			
Animal Laboratory				
(P. Hamosh)				
National Biomedical				
Research Foundation				
Programmer				
Computer				

* Members of the center.

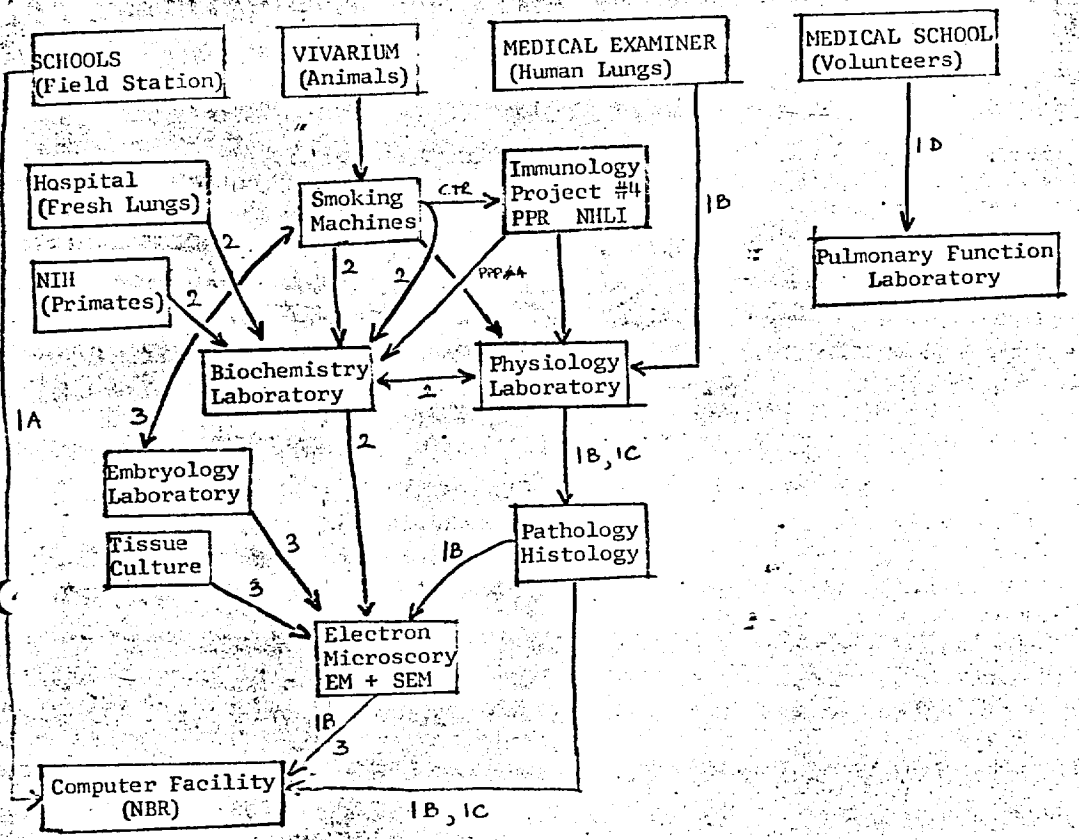
Figure 3.

OVERVIEW

P. HAMOSH M.D.

OVERVIEW

INTERACTION OF SOURCES AND LABORATORIES
IN THE DEVELOPMENTAL PROGRAM PROJECT



The Numbers Represent The Projects Described in the Proposal

FIGURE 4.